THERMOTOLERANT FUNGI ASSOCIATED
WITH TAIWAN HOT SPRINGS

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Abstract: The soil of hot springs in northern Taiwan sharing high temperatures of the springs was examined for fungi. Thermotolerant fungal species isolated included Aspergillus fumigatus Fresenius, Cephalosporiopsis imperfecta Peyronel, Chrysosporium tropicum Carmichael, and Humicola insolens Cooney and Emerson. One thermophilic fungal species, Humicola lanuginosa (Griffon and Maublanc) Bunce, was isolated from pigeon coprophilous material collected in Taipei during the time of the sulfur hot springs studies.

Introduction: Habitats of fungi are as diverse as is the anatomy of the achlorophillous thallophytes. Any object is subject to fungal attack or fungal association regardless of composition or structure. Temperature is a factor in survival and structural development of fungi, with the optimum temperature at 25 - 30 C for the best growth of most species (Cochrane, 1958). A few fungi grow well at higher temperatures but contradictions in terminology occur among investigators who isolated fungi from environments possessing high temperatures. Mycological studies have established a thermophilic fungus as a species with maximum temperature for growth at or above 50 C and a minimum growth temperature at 20 C, while minimum growth temperatures below 20 C the species is considered thermotolerant (Cooney and Emerson, 1964). More fungal species could be identified as thermophilic if the optimum temperature for growth is identified as above 40 C without establishing a minimum temperature limitation (Crisan, 1959).

The sulfur hot springs of northern Taiwan were investigated for the presence of fungi. Soil adjacent to the springs and sharing the springs high temperatures was collected for the isolation of fungi. Thermotolerant rather than thermophilic is a more acceptable label for the isolated fungal species. No attempt was made to establish the minimum, optimum and maximum temperatures for growth of each fungal isolate obtained from the hot springs soil. No soil collections were made at springs capped and diverted for commercial use at sulfur springs recreational areas. Soil collections were made only in undisturbed habitats at the source of the springs. All isolated species were commonly found in the hot spring soil.

Materials and Methods: Soil was collected from the banks of hot springs and streams leading from the spring source. The samples were always obtained from moist soil near the source of the spring. Four spring areas in northern Taiwan were selected for the mycological studies. Soil was placed in sterile plastic bags and transported to the laboratory for further examination. The sulfur hot springs selected for study and the accompanying water temperature included Peitou 95 C, Yangmingsan 110 C, Chiaoshi 47 C, and Chihsinshan 50 C. The collected soil adjacent to the water source had temperatures of 45 - 85 C and the soil was continually saturated with the sulfur spring water. All collection sites were within a 10 - 15 kilometer radius and north of Taipei.

Soil in 5 gram quantities was placed on yeast glucose agar plates and Sabouraud's maltose agar plates and placed in a 45 C incubator. Additional plates contained water saturated soil with powdered agar media sprinkled over the soil surface for an added nutrient source. A third group of agar plates was prepared with water suspensions of the soil spread on the agar surface in 3 ml quantities. Plates were incubated for one month with frequent additions of sterile distilled water to prevent desiccation at the selected incubation temperature. After sufficient growth, pure cultures were made of the recovered fungi. Species identification was determined according to the literature (Barnett and Hunter, 1972; Barron, 1968; Ellis, 1971; Gilman, 1957; Raper and Fennell, 1965; Rebell and Taplin, 1970).

Results: The highly specialized environment of the hot sulfur springs in Taiwan yielded four thermotolerant fungal species. Unique microscopic characteristics of the organisms greatly assist in species identification (Figure 1). The green colored conidiophores of Aspergillus fumigatus Fresenius are short, smooth walled, up to 300 μ in length by 5 - 8 μ in diameter, gradually enlarging upward and developing into an apical flask shaped vesicle. The vesicles are 20 - 30 μ in diameter, with sterigmata in one series appearing only on the upper half. Conidia are green in mass, echinulate, globose, 2.5 to 3.0 μ in diameter (Raper and Fennell, 1965).

Simple, slender conidiophores bearing two celled conidia in spherical heads are characteristic of Cephalosporiopsis imperfecta Peyronel. The genus is easily confused with the genera closely resembling the conidia of Cephalosporiopsis sp. (Barron, 1968; Booth, 1971; Guillemat and Montegut, 1957; Kamyschko, 1961; Sukapure and Thirumalachar, 1966). Records of the genus isolated from soil are rare (Guillemat and Montegut, 1957). The two celled phialospores gather in balls at the hyphal apex. Conidia are 12 - 14 μ x 1.5 - 2 μ in size, tapered at the ends.

Chrysosporium tropicum Carmichael grows well at 37 C (Carmichael, 1962; Rebell and Taplin, 1970). Aleuriospores are small, smooth walled and somewhat variable in size. The aleuriospores vary from 3 - 5 x 4 - 9 μ but generally are 3.5 - 4 x 6 - 7 μ in size (Carmichael, 1962). Spores are borne at hyphal tips, directly along the sides, or on lateral branches. Arthrospores also occur. Other

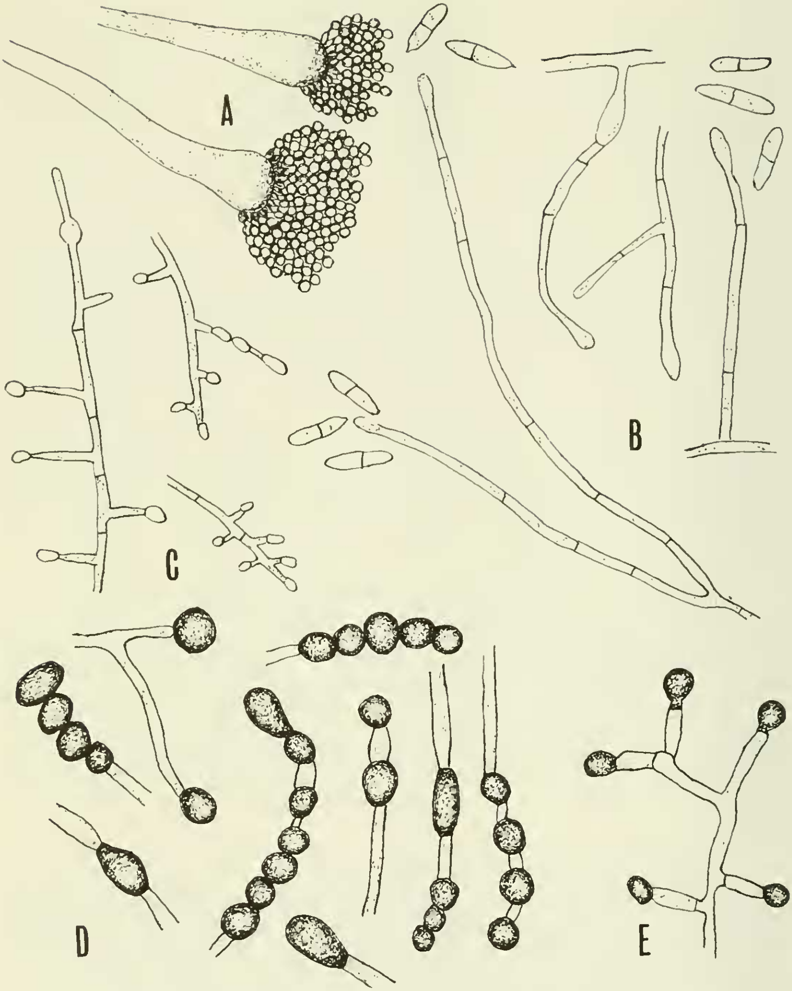

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Figure legend: a. *Aspergillus fumigatus*;
b. *Cephalosporiopsis imperfecta*;
c. *Chrysosporium tropicum*;
d. *Humicola insolens*;
e. *Humicola lanuginosa*.

Chrysosporium species are soil borne also but they do not grow at high temperatures.

Single aleuriospores of Humicola insolens Cooney and Emerson are borne terminally on hyphae or on short side branches (Cooney and Emerson, 1964). A few lateral branches produce chains of spores. Spores are globose, 7.5 - 12.5 μ in diameter, or flask shaped, 14 - 20 x 8 - 10 μ in size. Occasionally intercalary spindle shaped spores are formed. The thermophilic species found associated with pigeons is Humicola lanuginosa (Griffon and Maublanc) Bunce. The latter species has short aleuriophores developing at right angles to the hyphae. At times septations occur in these specialized branches. Spores are borne singly at the apex of aleuriophores, and they are smooth walled, somewhat wrinkled at maturity, 6 - 10 μ in diameter. The genus is easily confused with other genera that closely resemble the morphology of Humicola.

The heated habitat of sulfur springs in Taiwan yielded a few commonly found fungal species. Numerous studies recently reviewed in the literature (Crisan, 1973) identify various species found in heated habitats. Attention is now directed to physiology and biochemistry of the organisms in their selected ecological niches. Concepts attributing growth and survival at high temperatures are increasing yet it remains difficult to identify a habitat as the most suited natural occurring situation for a specific species.

Discussion: Hot springs have been previously examined for microorganisms that could survive high temperatures. Inhabitants include blue green algae (Copeland, 1936), bacteria (Allen, 1953), and Actinomycetes (Henssen, 1957) while the fungal isolates in the current study were obtained from the hot water saturated soil adjacent to the hot sulfur springs. It is interesting to note the variation in optimum temperature requirements in the literature that authors selected to identify species as thermophilous. The same fungal species has been identified as either thermotolerant or thermophilic depending on the author and the study. The former term infers that the species can survive high temperatures as well as low temperatures, while a thermophilic species is dependent on high temperatures for normal growth and development and lower temperatures would presumably cause stress to the species. The terminology has been further complicated with adjactives attached to terms such as the thermophiles that are facultative (Morrison and Tanner, 1924), stenothermal, eurithermal (Imsenecki and Solnzeva, 1945), microthermophilic, psychrotolerant, and orthothermophilic (Apinis, 1963).

Optimum temperatures for thermophilic species have been identified as being high or a number between 40 - 70 C selected by authors reviewed by Crisan (1964). The optimum temperature requirements for normal fungal growth and development appears more significant in identifying thermophilic from thermotolerant species, however, it is the minimum temperature that helps to separate the two terms. No optimum temperature requirement or tolerance studies were made with the Taiwan fungal isolates. A more limited definition of terms was used for this study, thus fewer species of fungi were considered thermophilic or heat loving. Reducing the number of species considered thermophilic brings greater continuity with previous authors on thermophilic fungi although attention in the literature is directed to the minimum temperature requirements. However, minimum temperature requirements seem less significant when the environment selected for study involves high temperatures.

An incubation temperature of 45 C was selected for this study between the 50 C maximum temperature set for thermophilic fungal growth by Cooney and Emerson (1964) and the 40 C optimum temperature for normal growth selected by Crisan (1959). The 50 C maximum temperature requirement also had a minimum temperature requirement for growth at or above 20 C. Thermotolerant fungi according to Cooney and Emerson were species with minimum requirements well below 20 C and with 50 C maximum. Each definition restricts the number of species included in the terms. In the controversy with Cooney and Emerson, Crisan considered his opponents minimum and maximum temperature requirements for identifying a thermophilic species as failing to distinguish between fungi that are truely thermophilic with mesophilic species that have an unusual heat tolerance yet grow below 20 C. Cooney and Emerson believed that the maximum and minimum temperatures were more easily established than optimum temperatures. Many fungi have an optimum temperature above 40 C yet still grow vigorously well below 20 C. For Cooney and Emerson it is difficult to distinguish between normal and abnormal growth of a species. Furthermore, if human and animal fungal pathogens were considered thermophilic, establishing normal growth in the dimorphic pathogens would add to the complexity of terminology. Cooney and Emerson believed Crisan, according to his definition, should include several additional species. The limited number of species considered thermophilic in both previous studies and the additional species identified as thermotolerant by Cooney and Emerson were also identified as thermophilic or thermotolerant in the Taiwan studies. Each previous study would be in agreement with Humicola lanuginosa as the thermophilic species isolated in Taiwan. Aspergillus fumigatus, Cephalosporiopsis imperfecta, Chrysosporium tropicum and Humicola insolens are thermotolerant to Cooney and Emerson, and thermophilic to Crisan. Regardless of terminology, with high temperatures and high quantities of dissolved minerals in the soil, it is most interesting to frequently isolate the species identified in the thermal sulfur springs of northern Taiwan.

Thermophilic and thermotolerant fungi have been isolated from self heating wood chips stored at a paper factory (Tansey, 1971). The presence of these fungi along with species of bacteria contribute to the heating and breakdown of organic material from which these saprobes obtain nutrients. Stored hay serves as a suitable habitat for the heat loving fungi (Bunce, 1961; Chang and Hudson, 1967; Rész, 1968), in addition to stored grains such as barley (Flannigan, 1969; Mulinge and Apinis, 1969), oats and wheat (Gilman and Barron, 1930), and corn (Awao and Mitsugi, 1973). The relationship between molds found on bin-burned grain and the production of heating was studied by several early investigators as noted by Gilman and Barron (1930).

Bagasse or crushed sugar extracted cane was found to contain thermophilic fungal species (Seabury et al., 1968). Fungal mycelium grew profusely at 60 C and above within collected stacks of oil palm kernels at oil mills in Nigeria (Eggins, 1964). Fresh pecans also serve as a good growth medium (Huang and Hanlin, 1975). Retting guayale is a habitat for Penicillium species suited for growth at high temperatures (Raper and Thom, 1968). Coprophilous material high in nitrogen sources also supports thermophilic fungal growth (Awao and Mitsugi, 1973; Cooney and Emerson, 1964; Crisan, 1964; Henssen, 1957; Lohr and Olsen, 1969; Waksman, et al., 1939). The partially decomposed nesting material of alligators (Tansey, 1973) and birds (Eicker, 1972; Frith, 1962; Noack, 1912) provides habitats for thermophilic fungi. Feathers and excreta in addition to nesting material of blackbirds, chickens (Cooney and Emerson, 1964), and Australian megapodes (Erith, 1959; Wetmore, 1933) were the subject of thermophilic fungal studies. Apinis and Pugh (1967) isolated 27 species of thermophilous fungi from nests of sandmartin, swallow, hedge sparrow, greenfinch, and reed bunting. Geothermal heat of pasture soil (Eggins and Malik, 1969), peat (Kuster and Locci, 1964), forest soil (Ward and Cowley, 1972), and alluvial soils (Apinis, 1963b; Awao and Otsuka, 1974; Craveri, et al., 1967; Maheshwari, 1968) creates additional natural habitats while man made niches of similar nature include mushroom compost (Fergus, 1964), coal spoil tips (Evans, 1971), city trash (Von Klopotek, 1962), and solid waste municipal compost systems (Kane and Mullins, 1973).

Some aspects of thermophilic fungal physiology were studied. Vitamins (Loginova, 1959) and amino acids (Morgan, 1972) were examined in nutritional requirement studies of thermophilic fungi when subjected to high temperatures in contrast to growth of fungi at reduced temperatures. Enzyme properties and modes of action were recently examined in thermophilic fungi. Enzyme systems and requirements studied included lipase (Bruszewski, et al., 1972; Liu, et al., 1973a, 1973b; Mumma, et al., 1970), β-glucosidase (Lusis and Becker, 1973), glucose-6-phosphate dehydrogenase (Broad, 1970). Additional nutritional studies have been conducted (Hedger and Hudson, 1974; Loginova, 1960), including pH requirements (Malik and Eggins, 1973) in order to further examine the ecological niche of heat loving fungal species.

Thermal soils and hot springs of Yellowstone National Park were examined for the existence of fungi at high temperatures (Tansey and Brock, 1971). The studies yielded Humicola lanuginosa and

Aspergillus fumigatus, two of the fungal species found in the Taiwan sulfur hot spring soil. Only thermotolerant fungal species were isolated from the sulfur springs, the thermophilic species was found in a pigeon loft in Taipei during a previous study (Volz and Yeh, 1976).

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